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EXAMINER	
TURNER, SHARON L	
ART UNIT	**PAPER NUMBER**
1649	

DATE MAILED: 04/14/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/207,649	LINDQUIST, SUSAN	
	Examiner	**Art Unit**	
	Sharon L. Turner	1649	

-- ***The MAILING DATE of this communication appears on the cover sheet with the correspondence address*** --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *22 October 2004*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,3,7-20,22 and 37* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,3,7-20,22 and 37* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)

2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)

3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.

4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.

5) ☐ Notice of Informal Patent Application (PTO-152)

6) ☐ Other: _____.

DETAILED ACTION

Continued Examination Under 37 CFR 1.114

1. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 2-9-06 has been entered.

2. The amendment filed 2-9-06 has been entered into the record and has been fully considered.

3. The text of Title 35 of the U.S. Code not reiterated herein can be found in the previous office action.

4. As a result of Applicant's amendment, all rejections not reiterated herein have been withdrawn.

Claim Rejections - 35 USC § 103

5. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

6. Claims 1, 3, 7, 9, 12-13, 15, 17-19 and 37 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Cordell et al., WO91/04339, 4 April 1991, Patino et al.,

Science, (1996 Aug 2) Vol. 273, No. 5275, pp. 622-6, Hughes et al., Proceedings of the National Academy of Sciences of the United States of America, (1996 Mar 5) Vol. 93, No. 5, pp. 2065-70, Rieger et al., Nature medicine, (1997 Dec. 1) Vol. 3, No. 12, pp. 1383-8, Hitzeman et al., US 4,775,622, 4 October 1988 and Chang et al., US 5,010,003 23 April 1991.

Cordell et al teach assays and reagents suitable for testing inhibitors of amyloid aggregation/amyloid deposition including the identification of agents that inhibit amyloid formation, see in particular Cordell, p. 4, line 9-p. 5, line 15 and claim 1, as claimed in claim 1. In particular the invention further teaches using modified beta-amyloid constructs including fusion proteins and viruses recombinantly constructed for expression of beta-amyloid peptides and in particular Abeta1-42 peptide for example, see in particular pp. 6-13, as claimed in claims 1 and 3. These constructs correspond to mammalian aggregate –prone amyloid proteins as noted by Applicants specification, see in particular p. 5, lines 26-27. In this respect Cordell particularly teaches amyloid fusion proteins labeled as noted in Figure 1 and pp. 10-11 comprising the 42 amino acid amyloid protein fused with the gene for ampicillin resistance, a drug-resistant detectable marker protein label, as in claims 1, 3, 7, 9, 12, 13 and 17. Cordell further notes that the amyloid products produced may be expressed in yeast and include beta-amyloid 1-42, see in particular p. 6, lines 5-30 and p. 7, line 13, p 10, lines 12- p. 11, lines 3-28. The methods include screening compounds for inhibition of aggregate fibril formation and the formation of amyloid aggregates or fibrillary material may be detected by Congo red staining which is indicative of amyloid fibrils and fibrillary material, see in particular p. 2,

lines 3-9, p. 6, lines 29-30, and p. 13, lines 20-36, as claimed in claims 1, 3, 7, 9, 12-13, 15, 17 and 37. Aggregates of the screening assay may further be detected by attachment of labeled antibodies such as antibodies labeled with fluorescent, enzymatic or radioactive (35S) labels, see in particular, pps. 14-15, especially p. 15, lines 5-6 and 19-20. In addition, the amyloid specific peptide may be detected using 35S-methionine-labeling of the peptides as they are produced in the cells, see in particular p. 21, line 4-p. 23, line 6, as in claims 17-19.

Thus, Cordell et al., teach a method of identifying a candidate substance that inhibits the aggregation of a mammalian aggregate-prone amyloid protein comprising: (a) contacting a cell that expresses a chimeric aggregate-prone amyloid protein comprising a mammalian aggregate-prone amyloid peptide with said candidate substance under conditions effective to allow aggregated amyloid formation; and (b) determining the ability of said candidate substance to inhibit the aggregation of the aggregate-prone amyloid protein as claimed. Although Cordell teaches that such expression may be achieved in a yeast cell, Cordell fails to particularly exemplify such expression in a yeast cell as noted in claim 1 and the Decision. However, Cordell further notes that detection via antibody recognition is a preferred method of detection. Yeast offer the advantage in this case that antibody to beta amyloid aggregates would be specific to chimeric aggregates of beta amyloid in the assay and not to aggregates of naturally occurring beta amyloid which is present in mammalian cells. Accordingly, the artisan would be further motivated to evaluate mammalian aggregation in yeast cells when detecting via antibody because the antibody signal would be solely from the test

peptide as the yeast do not provide an endogenous copy.

Patino et al., teach a cytoplasmically inherited genetic element in yeast termed [PSI+], which is a prion-like aggregate of the cellular protein Sup35 by differential centrifugation analysis and microscopic localization of a Sup35-green fluorescent protein chimeric fusion protein. Aggregation depended on the intracellular concentration and functional state of the chaperone protein Hsp104 in the same manner as did [PSI+] inheritance. The amino-terminal and carboxy-terminal domains of Sup35 contributed to the unusual behavior of [PSI+]. [PSI+] altered the conformational state of newly synthesized prion proteins, inducing them to aggregate as well, thus fulfilling a major tenet of the prion hypothesis. In particular, Patino's experimentation is one where protein aggregation is monitored directly in yeast cells, evidencing the art recognized principle that such cells are capable of providing for determining the ability of substances to inhibit (or promote) aggregation in yeast cells. The Sup 35 is a chimeric amyloid peptide that aggregates into beta pleated sheet formation. In this assay candidate substance Hsp104 or modified Sup35 were capable of affecting aggregation with contact in yeast cells. Accordingly, Patino exemplifies aggregation and inhibition of aggregation via contact in a yeast cell as claimed. However, the aggregate-prone amyloid peptide is not mammalian.

Hughes et al., teach a two-hybrid system as a model to study the interaction of beta-amyloid peptide monomers in yeast cells. Interaction of Abeta with itself was with fusion proteins created by linking the Abeta fragment to a LexA DNA-binding domain bait) and also to a B42 transactivation domain (prey). Protein-protein interactions were

measured by expression of these fusion proteins in yeast harboring lacZ (beta-galactosidase) and LEU2 (leucine utilization) genes under the control of LexA-dependent operators. Hughes concludes that the experimentation suggests that the Abeta molecule is capable of interacting with itself and aggregating in vivo in the yeast cell. Specifically, LexA protein fused to the Drosophila protein bicoid (LexA-bicoid) failed to interact with the B42 fragment fused to Abeta. Specificity was further shown by the finding that no significant interaction was observed in yeast expressing LexA-Abeta bait when the B42 transactivation domain was fused to an Abeta fragment with Phe-Phe at residues 19 and 20 replaced by Thr-Thr (AbetaTT), a finding that is consistent with in vitro observations made by others. When a peptide fragment bearing this substitution was mixed with native Abeta-(1-40), it inhibited formation of fibrils in vitro as examined by electron microscopy. The authors suggest that the yeast two-hybrid system can be used to study the interaction of Abeta monomers and to define the peptide sequences that may be important in nucleation-dependent aggregation. Accordingly, the authors use yeast cells for the study of aggregation properties particularly with mammalian aggregate prone amyloid protein.

In a similar article, Rieger et al., Nature medicine, (1997 Dec. 1) Vol. 3, No. 12, pp. 1383-8 teach that mammalian prion protein is similarly capably of aggregating with itself and inhibit aggregation of prion aggregates via competitive inhibition binding with laminin receptor protein in yeast. Prions are infectious mammalian proteins that cause transmissible spongiform encephalopathies. The pathogenic prion protein PrPSc converts its host encoded isoform PrPC into insoluble aggregates of PrPSc,

concomitant with pathological modifications. Rieger uses the yeast two-hybrid technology in Saccharomyces cerevisiae, to identify the 37-kDa laminin receptor precursor (LRP) as capable of interacting with the cellular prion protein PrPC. Mapping analysis of the LRP-PrP interaction site in S. cerevisiae revealed that PrP and laminin share the same binding domain (amino acids 161 to 180) on LRP. The LRP-PrP interaction was confirmed in vivo in insect (Sf9) and mammalian cells (COS-7). Accordingly, Rieger et al., teach the determination of LRP or laminin to inhibit aggregation with mammalian PRP in yeast cells.

Hitzeman et al., further teach expression, processing and secretion of heterologous protein by yeast. In particular the expression in yeast is noted to be advantageous in that the method allows for high recovery of discrete product unaccompanied by unwanted presequence or other artifact of expression, see in particular column 1, lines 10-16. In addition high quantities of the protein in mature form are secreted into yeast media without the need for further purification steps such as cell lysis, see in particular column 1, lines 28-37 and column 2, lines 30-56.

Chang et al., similarly teach expression, processing and secretion of heterologous protein by yeast and also teach use of yeast homologous signals to secrete heterologous proteins. In addition to the advantages of high level expression in yeast without interfering amounts of unwanted presequence or other artifact of expression, use of the signal peptide coding portion of a signal homologous to yeast from the yeast gene, in some cases provides a yield of mature protein that is higher than that obtained when the protein is expressed with its natural signal peptide. Further

it is noted that the fidelity of processing is also sometimes improved, see in particular column 1, lines 15-64.

Thus one of skill in the art would be motivated by Patino, Hughes, Rieger, Chang and Hitzeman to modify the screening assay of Cordell so as to express the mammalian aggregate-prone proteins in yeast. Patino, Hughes and Rieger teach that yeast cells produce recombinant peptides including mammalian and allow for the evaluation of aggregate formation of such peptides in yeast cells. Hizeman and Chang also teach the ability to produce recombinant mammalian peptides in yeast. While Hizeman and Chang speak to advantages of yeast in mediating soouble expression they also note that peptide production need not be so modified for soluble expression. Yeast are largely used for recombinant peptide production because of their ease of culture, fast growth, and high yield. One of skill in the art would have expected positive results using this modification given the suggestion of Cordell, Patino, Hughes and Rieger to achieve such expression in yeast and to evaluate aggregation in the yeast cells. Hizeman and Chang further reiterate the widely accepted principle of recombinant peptide production in yeast, even without a modification that further provides the ability to test aggregation in such media directly. Thus, the cumulative reference teachings render the claimed invention obvious to one of ordinary skill in the art.

Applicant's argue as set forth In the response of 10-22-04, pages 1-4. In particular Applicant's refer to the deficiency of the picking and choosing or selection of portions referred to by the Board in its decision of 2-27-04, in particular pp. 8-10 and reference to "Other issue" at p. 13, particular teachings of the noted references and

cites In re Dow Chemicals Co., noting obviousness requires the combination as suggested or motivated must yield the claimed invention. In particular Applicants argue that the combination fails to arrive at the invention because claim 1 requires that the method be carried out in a yeast cell. Accordingly, Applicant's assert obviousness is not found.

Applicants arguments filed 10-22-04 have been fully considered but are not persuasive. In particular, claim 1 does not provide the limitation "in a yeast cell" anywhere in the claim. The claim merely requires "contacting a yeast cell that expresses ...the protein...with said candidate substance...". There is further no requirement that the aggregation be determined inside the yeast cell. In fact the guidance provided via the specification appears to show that at least some embodiments of the invention are *not* in a yeast cell as suggested for example at pp. 28, lines 10-29 noting "5.1.1.3. PrP Purification" and "5.2.1.4. Cell free PrP Conversion", see also results p. 29-34 where the precipitation or aggregation event appears to be outside of a yeast cell. Each of the Cordell, Hitzeman and Chang references note that the expression of recombinant proteins may be achieved in yeast cells and Hitzeman and Chang motivate via particularly noted advantages for expression in yeast cells. Nevertheless, this does not preclude that the expression of the proteins involves an intracellular phase where the protein may be contacted while inside the cell and prior to secretion. Accordingly the limitations are met and the combination as suggested arrives at the claimed invention. Rejection is maintained.

Applicant's amendment of 2-9-06 now inserts the limitation "in a yeast cell" with

respect to the claims. Applicants argue that therefore the invention is defined over the art of record. In particular, Applicants argue that Cordell does not describe an assay that uses a yeast cell expressing chimeric protein to evaluate aggregation in the yeast cell per se. Applicants thus conclude that Hitzeman and Chang do not supplement and attempt to distinguish that Hitzeman and Chang are on point to secretion and thus provide no expectation of success in achieving or evaluating aggregation in yeast cells.

Applicant's arguments have been fully considered but are not persuasive. While it is true that Cordell teaches achieving and evaluating aggregation in vitro, Cordell makes clear that the invention is inclusive of cellular aggregation and evaluation, see in particular p. 3-4, also Summary pp. 4-5 noting that the assay is in "a cellular environment," p. 4, line 24, and the "use of immunological reagents to detect the formation of preamyloid protein aggregation in the cell lines," p. 4, lines 27-29. One noted embodiment is where vaccinia vectors provide the chimeric (or mutated) amyloid, and another is where such production is in yeast cells, see pp. 10-11, 15-18 for example. Accordingly, Cordell does teach where the assay may be carried out in yeast. As Cordell does exhibit this teaching Hitzeman and Chang do still supplement. They explicitly teach that yeast are capable of high level expression of mammalian peptides such as chimeric amyloid, they are easy to grow and produce large quantities of recombinant peptide. While they enable secretion when included with a signal sequence, such is not necessarily required. One need not modify or add the signal sequence for secretion. Instead, as taught by Cordell, one may assay in the cell, as long as the cell is capable of peptide production and Hitzeman and Chang make it clear

that yeast are suitable for high level expression and peptide production in yeast cells of recombinant mammalian peptides. Cordell further notes that detection via antibody recognition is a preferred method of detection. Yeast offer the advantage in this case that antibody to beta amyloid aggregates would be specific to chimeric aggregates of beta amyloid in the assay and not to aggregates of naturally occurring beta amyloid which is present in mammalian cells. Accordingly, the artisan would be further motivated to evaluate mammalian aggregation in yeast cells when detecting via antibody because the antibody signal would be solely from the test peptide as the yeast do not provide an endogenous copy. cumulative reference teachings anticipate the claimed invention and the artisan is provided motivation to utilize yeast cells as the yeast cells provide for the advantage of cell culture systems capable of expressing recombinant mammalian peptides and further offer the advantage that chimeric aggregates of amyloid may be detected in the absence of aggregation of endogenous amyloid that is not present in yeast cells. Thus, all detection of amyloid aggregate is indicative of the chimeric construct.

Nevertheless, Patino, Hughes and Rieger also motivate to evaluation of aggregation in yeast cells. In Patino the evaluation is of a yeast aggregate prone amyloid peptide, in Hughes and Rieger the evaluation is of mammalian aggregate prone amyloid peptide. Each is evaluated in yeast cells via recombinant production of chimeric constructs. Accordingly, the cumulative reference teachings render the claimed invention obvious to the artisan.

7. Claim 8, 17-18 and 20 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Cordell et al., WO91/04339, 4 April 1991, Patino et al., Science, (1996 Aug 2) Vol. 273, No. 5275, pp. 622-6, Hughes et al., Proceedings of the National Academy of Sciences of the United States of America, (1996 Mar 5) Vol. 93, No. 5, pp. 2065-70, Rieger et al., Nature medicine, (1997 Dec. 1) Vol. 3, No. 12, pp. 1383-8, Hitzeman et al., US 4,775,622, 4 October 1988 and Chang et al., US 5,010,003 23 April 1991 as set forth above and further in view of Chalfie et al., Science, (1994 Feb 11), 263(5148):802-805.

Cordell, Patino, Hughes, Rieger, Hitzeman, and Chang are rendered obvious as set forth above.

While the references teach and/or motivate to various fusion proteins comprised of mammalian aggreggate-prone amyloid proteins expressed with various detectable marker proteins and/or labels in yeast cells, the references fail to teach fusion with the marker protein green fluorescent protein (gfp) as claimed in claim 8 and detection of the fluorophore/chromophore label of green fluorescent protein as claimed in claims 17-18 and 20.

Chalfie et al., teach green fluorescent protein (gfp) as a marker for gene expression. In particular, when produced in either prokaryotic or eukaryotic cells, gfp can be used to monitor gene expression and protein localization using the simplistic method of fluorescence microscopy, see in particular abstract and Figure 3.

Thus one of skill in the art would be motivated by Chalfie et al., to modify the fusion proteins of Cordell, Patino, Hughes or Rieger such that fluorescence via green

fluorescent protein could be used to monitor protein expression in the cell culture assay. This modification provides the advantages of monitoring the aggregate expression of the mammalian aggregate-prone amyloid proteins using the simple method of light fluorescence. This would be particularly useful where aggregation was in culture media as expressed in yeast. Such media could be directly analyzed by microscopy without further need of processing such as via detection by staining with Congo Red or with labeled antibodies. One of skill in the art would have expected positive results using this modification given the suggestion of Chalfie to achieve monitoring of gene expression using fluorescence as opposed to more complex methods of detection such as with Congo Red staining or antibody based detection. Thus, the cumulative reference teachings render the claimed invention obvious to one of ordinary skill in the art.

Applicant's argue as set forth In the response of 10-22-04, page 4. In particular, Applicants argue that the combination fails to arrive at the invention because claim 1 requires that the method be carried out in a yeast cell. Accordingly, Applicant's assert obviousness is not found.

Again, applicants arguments filed 10-22-04 have been fully considered but are not persuasive. In particular, claim 1 does not provide the limitation "in a yeast cell" anywhere in the claim. The claim merely requires "contacting a yeast cell that expresses ...the protein...with said candidate substance...". There is further no requirement that the aggregation be determined inside the yeast cell. In fact the guidance provided via the specification appears to show that at least some

embodiments of the invention are *not* in a yeast cell as suggested for example at pp. 28, lines 10-29 noting "5.1.1.3. PrP Purification" and "5.2.1.4. Cell free PrP Conversion", see also results p. 29-34 where the precipitation or aggregation event appears to be outside of a yeast cell. Each of the Cordell, Hitzeman and Chang references note that the expression of recombinant proteins may be achieved in yeast cells and Hitzeman and Chang motivate via particularly noted advantages for expression in yeast cells. Nevertheless, this does not preclude that the expression of the proteins involves an intracellular phase where the protein may be contacted while inside the cell and prior to secretion. Chalfie provides for the further advantage of the marker gene of green fluorescent protein. Accordingly the limitations are met and the combination as suggested arrives at the claimed invention. Rejection is maintained.

Applicants argue in the 2-9-06 response that the combination with Chalfie would result in an assay in which protein was secreted into the cell culture media. However, as noted above, one need not modify the protein via inclusion of the signal sequence to achieve secretion. Accordingly, Cordell, Patino, Hughes and Rieger are inclusive of intracellular aggregation assays and therefor render obvious such intracellular evaluation.

8. Claims 7 and 10-11 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Cordell et al., WO91/04339, 4 April 1991, Patino et al., Science, (1996 Aug 2) Vol. 273, No. 5275, pp. 622-6, Hughes et al., Proceedings of the National Academy of Sciences of the United States of America, (1996 Mar 5) Vol. 93, No. 5, pp. 2065-70, Rieger et al., Nature medicine, (1997 Dec. 1) Vol. 3, No. 12, pp. 1383-8, Hitzeman et

al., US 4,775,622, 4 October 1988 and Chang et al., US 5,010,003 23 April 1991 as set forth above and further in view of Tikhonenko et al., Oncogene, (1995 Oct. 19), 11(8):1499-508.

Cordell, Patino, Hughes, Rieger, Hitzeman, and Chang render obvious as set forth above.

While the references teaches various fusion proteins comprised of aggreggate-prone amyloid proteins expressed with various detectable marker proteins, the references fails to teach detection of gene expression via fusion with the marker protein glucocorticoid hormone receptor protein.

Tikhonenko et al., teach conditional mutants expressing v-myc as a fusion protein with the glucocorticoid receptor and the retroviral Gag polyprotein. As noted by Tinkhonenko et al., the glucocorticoid receptor element is used as a marker protein for inducible expression via the presence or absence of glucocorticoids such as dexamethasone. Thus, the construct (GRIM) is only capable of transforming exbryo cells in the presence of glucocorticoids.

Thus one of skill in the art would be motivated by Tikhonenko to modify the fusion proteins of Cordell, Patino, Hughes or Rieger such that the glucocorticoid receptor could be used as a marker protein to both monitor and induce protein expression in the cell culture assay. This modification provides the advantages of regulating aggregate-prone amyloid protein expression using the simple method of induction via addition of glucocorticoids such as dexamethasone. One of skill in the art would have expected positive results using this modification given the suggestion of

Tikhonenko to achieve regulated peptide expression using the glucocorticoid receptor as the marker protein in the aggregate-prone amyloid fusion. It would be further advantageous to regulate expression of the amyloid protein so that the assay could be easily manipulated to test particular aggregate forming conditions. Such provides the ability to induce peptide expression at a discrete and chosen time so as to more closely monitor and test any particular candidate substance's ability to inhibit amyloid aggregate formation. Thus, the cumulative reference teachings render the claimed invention obvious to one of ordinary skill in the art.

Applicant's argue as set forth In the response of 10-22-04, page 4-5. In particular, Applicants argue that the combination fails to arrive at the invention because claim 1 requires that the method be carried out <u>in a yeast cell</u>. Accordingly, Applicant's assert obviousness is not found.

Again, applicants arguments filed 10-22-04 have been fully considered but are not persuasive. In particular, claim 1 does not provide the limitation "in a yeast cell" anywhere in the claim. The claim merely requires "contacting a yeast cell that expresses ...the protein...with said candidate substance...". There is further no requirement that the aggregation be determined inside the yeast cell. In fact the guidance provided via the specification appears to show that at least some embodiments of the invention are *not* in a yeast cell as suggested for example at pp. 28, lines 10-29 noting "5.1.1.3. PrP Purification" and "5.2.1.4. Cell free PrP Conversion", see also results p. 29-34 where the precipitation or aggregation event appears to be outside of a yeast cell. Each of the references note that the expression of recombinant

proteins may be achieved in yeast cells and Hitzeman and Chang motivate via particularly noted advantages for expression in yeast cells. Nevertheless, this does not preclude that the expression of the proteins involves an intracellular phase where the protein may be contacted while inside the cell and prior to secretion. Cordell motivates to this choice. The artisan futher recognizes the advantage of yeast cell expression when detection is via antibody to mammalian peptide. Further Patino, Hughes and Rieger motivate to evaluation of aggregation in yeast. Tihenko provides for the further advantage of the marker gene of the glucocorticoid receptor element. Accordingly the limitations are met and the combination as cumulatively suggested arrives at the claimed invention. Rejection is maintained.

Applicants argue in the 2-9-06 response that the combination with Tihenko would result in an assay in which protein was secreted into the cell culture media. However, as noted above, one need not modify the protein via inclusion of the signal sequence to achieve secretion. Accordingly, Cordell, Patino, Hughes and Rieger are inclusive of intracellular aggregation assays in yeast and therefor render obvious such intracellular evaluation.

9. Claim 16 stands rejected under 35 U.S.C. 103(a) as being unpatentable over Cordell et al., WO91/04339, 4 April 1991, Patino et al., Science, (1996 Aug 2) Vol. 273, No. 5275, pp. 622-6, Hughes et al., Proceedings of the National Academy of Sciences of the United States of America, (1996 Mar 5) Vol. 93, No. 5, pp. 2065-70, Rieger et al., Nature medicine, (1997 Dec. 1) Vol. 3, No. 12, pp. 1383-8, Hitzeman et al., US 4,775,622, 4 October 1988 and Chang et al., US 5,010,003 23 April 1991 as set forth

above and further in view of Nordtstedt et al., J. of Biol. Chem., (1994 Dec 9), 269(49):30773-6.

The aforementioned references render obvious as set forth above.

While the references teach various methods of detecting aggregate formation of aggreggate-prone amyloid proteins, the references fail to teach analysis of fibril formation via protease resistance as in claim 16.

Nordstedt et al., teach that Abeta peptide develops protease resistance in association with its polymerization into amyloid fibrils, see in particular Title and Abstract.

Thus one of skill in the art would be motivated by Nordstedt to determine the ability of the candidate substance to inhibit aggregation by assessing the aggregate-prone amyloid proteins aggregate formation as detected by increased protease resistance. This modification provides the advantage of monitoring the aggregate formation as it is expressed. One of skill in the art would have expected positive results using this modification given the suggestion of Nordstedt et al., to assess aggregate fibril formation as a function of protease resistance, a known indicator of fibril formation. Thus, the cumulative reference teachings render the claimed invention obvious to one of ordinary skill in the art.

Applicant's argue as set forth In the response of 10-22-04, page 5. In particular, Applicants argue that the combination fails to arrive at the invention because claim 1 requires that the method be carried out <u>in a yeast cell</u>. Accordingly, Applicant's assert obviousness is not found.

Again, applicants arguments filed 10-22-04 have been fully considered but are not persuasive. In particular, claim 1 does not provide the limitation "in a yeast cell" anywhere in the claim. The claim merely requires "contacting a yeast cell that expresses ...the protein...with said candidate substance...". There is further no requirement that the aggregation be determined inside the yeast cell. In fact the guidance provided via the specification appears to show that at least some embodiments of the invention are *not* in a yeast cell as suggested for example at pp. 28, lines 10-29 noting "5.1.1.3. PrP Purification" and "5.2.1.4. Cell free PrP Conversion", see also results p. 29-34 where the precipitation or aggregation event appears to be outside of a yeast cell. Each of the Cordell, Hitzeman and Chang references note that the expression of recombinant proteins may be achieved in yeast cells and Hitzeman and Chang motivate via particularly noted advantages for expression in yeast cells. Nevertheless, this does not preclude that the expression of the proteins involves an intracellular phase where the protein may be contacted while inside the cell and prior to secretion. Nordstedt teach that Abeta peptide develops protease resistance in association with its polymerization into amyloid fibrils thereby suggesting the advantage of assessing aggregate formation via protease resistance. Accordingly the limitations are met and the combination as suggested arrives at the claimed invention. Rejection is maintained.

Applicants argue in the 2-9-06 response that the combination with Norstedt would result in an assay in which protein was secreted into the cell culture media. However, as noted above, one need not modify the protein via inclusion of the signal

sequence to achieve secretion. One could produce the peptides without such signal and evaluate aggregation in the yeast cell. Accordingly, Cordell, Patino, Hughes and Rieger are inclusive of intracellular aggregation assays in yeast and therefor render obvious such intracellular evaluation.

10. Claims 14 and 22 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Cordell et al., WO91/04339, 4 April 1991, Patino et al., Science, (1996 Aug 2) Vol. 273, No. 5275, pp. 622-6, Hughes et al., Proceedings of the National Academy of Sciences of the United States of America, (1996 Mar 5) Vol. 93, No. 5, pp. 2065-70, Rieger et al., Nature medicine, (1997 Dec. 1) Vol. 3, No. 12, pp. 1383-8, Hitzeman et al., US 4,775,622, 4 October 1988 and Chang et al., US 5,010,003 23 April 1991 as set forth above and further in view of Patino et al., Science (August 1996), 273:622-626.

The aforementioned references render obvious as set forth above.

Cordell, Patino, Hughes and Rieger teaches various methods of assaying candidate substances that inhibit aggregation of aggregate-prone amyloid proteins. Hitzeman and Chang also teach expression in yeast for recombinant production of amyloid proteins. The cumulative references do not assay as recited where specifically the assay is with the N-terminal domain of Sup35 is replaced by amino acids 1-42 of beta amyloid or the yeast cell overexpresses Hsp104 as claimed in claims 14 and 22. However, Patino is on point to this construct.

Patino et al., teach that Sup35 is a non-mammalian (yeast) homologue of prion protein, an amyloid-like peptide that exhibits peptide aggregation similar to the formation of amyloid fibril-like aggregates in the brain and causes neurodegenerative disease, see

in particular Abstract and p. 622, columns 1-2. Patino et al., further teach that Hsp104 overexpression in yeast cells is capable of converting Sup35 from aggregating form [PSI+] to non-aggregating form [psi-]. In addition, Patino notes that the N-terminal domain is not essential and is required only for the propagation of [PSI+] aggregating prion-like form, see in particular column 2, lines 1-16. The mechanism of switch between aggregating and non-aggregating form is argued to be the inheritance of a self-perpetuating alteration in the conformation of Sup35, which is initiated by the NH2-terminal domain and impairs the ability of the COOH-terminal domain to function in translation, see in particular p. 622, column 2, lines 16-28. This mechanism is supported by the direct physical evidence of Patino as noted by the insolubility of Sup35 in aggregating [PSI+] cells and the ability of Hsp104 to convert to the non-aggregating [psi-] form, see in particular, pp. 622-625.

While the mechanisms of amyloid aggregation are unknown, Cordell motivates the artisan to test candidate substances for inhibition in order to determine those factors and mechanisms that regulate and are essential to amyloid aggregate formation. Patino notes that particular constructs are themselves inhibitors. Thus while Patino is not noted as a screening method the experimentation steps are the same as the recited screening method. One of skill in the art would be motivated by the cumulative references to test if the same mechanisms which regulate yeast prion aggregation in yeast cells are capable of regulating amyloid aggregate formation in yeast cells. Patino thus motivates the artisan to substitute beta-amyloid for that portion of the Sup35 protein that has been identified as capable of regulating prion-like formation to test for

its ability to regulate amyloid aggregate formation in yeast cells. To such end it would be the N-terminus of Sup35 that would be replaced with beta amyloid in order to determine if aggregate formation could occur in correlation with [PSI+] heritable form. Similarly, one of skill in the art would be motivated to perform such testing in yeast cells over-expressing Hsp104, such that the Hsp104 peptide expression could be tested as a candidate agent capable of inhibiting amyloid and/or prion aggregate formation in yeast cells either expressing Sup35 or Sup35 modified such that the heritable portion was replaced with beta amyloid. One of skill in the art would have expected the modifications to provide the ability to test if amyloid aggregation was similarly regulated as in yeast Psi+ determinant. Given the teachings of such screening methods, the ability to express such modified proteins in yeast and the understanding of yeast prion/Sup35 aggregate formation as noted by Patino, the artisan would be motivated to modify the assay to test if the mechanisms determined to regulate yeast prion aggregate formation in yeast are the same or different as aggregate formation in yeast with amyloid aggregates. The cells could easily be manipulated to overexpress Hsp104 as noted by Patino and to express N-terminal beta-amyloid modified Sup35 as taught by Patino. One of skill in the art would be further motivated to modify the screening method given the long need to discover those cellular mechanisms that regulate amyloid formation in vitro and in vivo. Whether the screening assay indicated that the regulation of amyloid aggregation was either the same as or different from yeast Sup35 would not be of particular concern. Regardless of the outcome, the results would further the artisan's knowledge as to the similarity and or difference between prion

aggregate formation in yeast as compared to amyloid aggregate formation in yeast. Thus, the cumulative reference teachings render the claimed invention obvious to one of ordinary skill in the art.

Applicant's argue as set forth In the response of 10-22-04, page 6. In particular, Applicants argue that the combination fails to arrive at the invention because claim 1 requires that the method be carried out in a yeast cell. Accordingly, Applicant's assert obviousness is not found..

Again, applicants arguments filed 10-22-04 have been fully considered but are not persuasive. In particular, claim 1 does not provide the limitation "in a yeast cell" anywhere in the claim. The claim merely requires "contacting a yeast cell that expresses ...the protein...with said candidate substance...". There is further no requirement that the aggregation be determined inside the yeast cell. In fact the guidance provided via the specification appears to show that at least some embodiments of the invention are *not* in a yeast cell as suggested for example at pp. 28, lines 10-29 noting "5.1.1.3. PrP Purification" and "5.2.1.4. Cell free PrP Conversion", see also results p. 29-34 where the precipitation or aggregation event appears to be outside of a yeast cell. Each of the Cordell, Hitzeman and Chang references note that the expression of recombinant proteins may be achieved in yeast cells and Hitzeman and Chang motivate via particularly noted advantages for expression in yeast cells. Nevertheless, this does not preclude that the expression of the proteins involves an intracellular phase where the protein may be contacted while inside the cell and prior to secretion. Patino teach as noted in the response Sup35 as a homologue of prion

protein and Hsp104 over expression in yeast cells as suitable for screening candidates for conversion of Sup35 from aggregating to non-aggregating form thereby providing motivation to screen Sup35 in Hsp104 over expressing cells. Accordingly the limitations are met and the combination as suggested arrives at the claimed invention. Rejection is maintained.

Applicants again argue in the 2-9-06 response that the combination would result in an assay in which protein was secreted into the cell culture media. However, Patino is on point to expression in yeast and not to secreted peptide production. Similarly Cordell, Hughes and Rieger as noted above are on point to yeast expression. Chang and Hizeman still supplement as they recognize that one need not modify the protein via inclusion of the signal sequence to achieve secretion. One could produce the peptides without such signal and evaluate aggregation in the yeast cell. Accordingly, Cordell, Patino, Hughes and Rieger are inclusive of intracellular aggregation assays in yeast and therefor render obvious such intracellular evaluation via specific recombinants of yeast peptide where the aggregating portion is substituted with aggregating amyloid. Again, Cordell motivates to evaluation of amyloid aggregates with antibody detection. When expressed in yeast, detection via this procedure is advantageous as the yeast do not contain endogenous copy. Therefore rejection is maintained.

Status of Claims

11. No claims are allowed.

Conclusion

12. Any inquiry of a general nature or relating to the status of this general application should be directed to the Group receptionist whose telephone number is (703) 308-0196.

Papers relating to this application may be submitted to Technology Center 1600, Group 1640 by facsimile transmission. The faxing of such papers must conform with the notice published in the Official Gazette, 1096 OG 30 (November 15, 1989). Should applicant wish to FAX a response, the current FAX number for Group 1600 is (703) 872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Sharon L. Turner, Ph.D. whose telephone number is (571) 272-0894. The examiner can normally be reached on Monday-Thursday from 7:00 AM to 5:00 PM. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Janet Andres can be reached at (571) 272-0867.

Sharon L. Turner, Ph.D.
April 13, 2006

SHARON TURNER, PH.D.
PRIMARY EXAMINER